REMARKS

Claims 1 and 5 are amended. Claims 4 and 6-10 are canceled. Claims 1-3 and 5 remain in the application.

Amendments to claims 1 and 5: Claim 1 is amended to recite a method executable by "an automated microscopy system" for measuring cell activity represented in an image of cells treated with an agent comprising "the automated microscopy system-executed steps of" segmenting, separating, and measuring. An embodiment of such a method is described and illustrated in this application. See, for example, FIGS. 1 and 29, and the accompanying descriptions. Claim 4 is amended to recite "cellular compartments" instead of "cellular material", which complies with the antecedent basis of claim 1.

Rejection of claims 1-5 under 35 USC 101: Claims 1-5 are rejected under 35 USC 101 as being directed to non-statutory subject matter. The rejection is moot with respect to claim 4, which is canceled. The rejection is traversed with respect to claims 1-3 and 5 for the following reasons.

It has recently been held the machine-or-transformation test of In re Bilski is not solely determinative of compliance of claimed subject matter with 35 USC 101, although it can be a useful tool for investigation of patentability. In this regard, the applicants respectfully submit that the subject matter of claims 1-3 and 5 in fact satisfies both prongs of the two-step test of In re Bilski.

First, independent claim 1 recites a method that is executable by "an automated microscopy platform". Further, the amendment of claim 1 now limits the method to one including "the automated microscopy platform-executed steps of" of segmenting, separating, and measuring.

Second, the claimed method is directed to methodology subject matter that transforms an image from one state to another. In this regard, for example, FIG. 7 illustrates an image of biological material that has been tessellated. Tessellation transforms images of overlapping cellular compartments by cutting them apart in order to improve measurement fidelity. See paragraph [0060] of the specification in this regard. The final step of claim 1 explicitly recites such a transformation.

Accordingly, the applicant respectfully submits that claims 1-3 and 5 are directed to patentable subject matter under the Bilski test.

Rejection of claims 4 and 5 under 35 USC 112, second paragraph: Claims 4 and 5 are rejected under 35 USC 112, second paragraph, for failing to particularly point out and distinctly claim the subject matter which is regarded as the invention. It is respectfully submitted that the cancellation of claim 4 removes the basis for this rejection.

Rejection of claims 1-3 and 6-8 for obviousness: Claims 1-3 and 6-8 are rejected for obviousness over US 6416959 ("Giuliano") in view of US 5528703 ("Lee") and Analytical Cellular Pathology, 21, p 71-86, 2000 ("Sudbo").The rejection is moot with respect to claims 6-8, which are canceled. The rejection is traversed with respect to claims 1-3 for the following reasons.

Overview

Claims 1-3 and 5 are directed to measurement of cell activity using magnified images of cellular material exposed to an agent. A magnified image is segmented and separated into compartments. Then intensities of cellular material in two or more components of a cell in the segmented, compartmentalized image are measured. Translocation of cellular material between first cellular compartments and second cellular compartments caused by the agent is measured by a ratio of measured intensity of a cellular compartment to a sum of measured intensities of the cellular material in two or more cellular compartments.

The combination of Giuliano with Lee and Sudbo does not satisfy the prima facie requirements of obviousness:

Claim 1 as amended now includes:

> "determining, in the segmented, separated image, translocation of cellular material between first cellular compartments and second cellular compartments caused by the agent, by:
>
> measuring a first intensity of the cellular material in a first cellular compartment of a cell;
>
> measuring a second intensity of the cellular material in a second cellular compartment of the cell; and,
>
> determining a fractional localized intensity of the cellular material in the first cellular compartment according to a ratio of the first intensity to a sum of at least the first and second intensities."

As conceded by the examiner, the combination of Giuliano with Lee and Sudbo does not show measurement of fractionalized intensity. Accordingly, claims 1-3 and 5 are not obvious of this combination. Accordingly, this rejection should be withdrawn.

Rejection of claims 4-5 and 9-10 for obviousness: Claims 4-5 and 9-10 are rejected for obviousness over Giuliano in view of Lee and Sudbo, and further in view of Pharmaceutical Research, Vol. 16, No. 2, pp. 327-332, 1999 ("Pohl").The rejection is moot with respect to claims 4, 9, and 10, which are canceled. The rejection is traversed with respect to claims 1-3 and 5 for the following reasons.

At p. 328, col. 2, bottom paragraph, Pohl states: "Identification of ROI (airspace, interstitium, type II cells, macrophages, and capillaries) was done by first locating the ROI on the red (RB) channel (7), and then masking the identified area. The mask was overlaid on the green channel image (probe), to identify the ROL." However, Pohl does not describe any methods of segmentation or separation, and so it is as likely that the segmentation was performed manually as by automated means.

Figure 3 of Pohl reports data on ROIs "interstitium" and "airspace," and Table 1 reports "Interalveolar Septal Thickness," which corresponds to the interstitium (see Fig. 1 of Pohl). Thus, these regions are not isolated objects or cells, but rather regions of tissue containing many cells and extracellular matrix. Accordingly, segmentation of intra-object components or cellular compartments was not carried out. Therefore, Pohl does not teach or suggest "determining, in the segmented, separated image, translocation of cellular material between first cellular compartments and second cellular compartments".

Pohl describes the calculations of fractional fluorescence on page 330, col. 2, at lines 1-6: "The average intensity and area of each mask on the green channel was calculated and recorded. Fraction of total fluorescence in each ROI was calculated by dividing the fluorescence from each ROI ([area in pixels] X [average intensity per pixel]) by the total fluorescence ([total area in pixels] X [average intensity per pixel])". But, as explained in the Background section of the application, this introduces an area-dependent error in the measure of translocation.

Relatedly, fractional localized intensity of cellular compartments (FLIC) precisely measures cellular translocation (redistribution) of components that are conserved over the time course of the experiment (none added to the cell or taken away). FLIC measurement requires image segmentation of the cellular compartments and calculation of the fractional localized intensity of the intracellular distributions. An exemplary embodiment of FLIC measurement is given by equation (1) and described in paragraph [0071]of this application. In contrast, in the prior art, there is no preference given to whether a difference or fraction is calculated, the fraction is calculated incorrectly, and accurate cellular image segmentation is not performed (see US 6,416,959 Fig. 10 and US 5,989,835 Fig. 8 for cellular masks

comprising a small ring around the nucleus that sample a small portion of the cytoplasm, rather than an entire cytoplasmic region). The "fraction" in these patents is a ratio of the averages of the intensities in the subcellular image segments, which introduces the area-dependent error described in the application: "Note that direct integration over the compartment image segments is preferred over average compartment intensity ratios or differences used in US 5,989,835 *because a ratio of areas causes a bias factor that confounds direct interpretation* unless $N_c = N_n = N_m$, which can only be artificially achieved by discarding a majority of the cytoplasm and nuclear signal because typically Nc,Nn > Nm" (US 2007/0016373 A1, page 8, paragraph [0071], italics added for emphasis). Thus, the invention recited in claim 1 is designed to precisely measure the translocation of conserved intracellular components – those that change location in response to cellular stimuli (such as NFkB) but are not gained or lost in the cell volume as a whole – whereas the prior art describes imprecise methods.

Thus, Pohl does not teach FLIC and the claims are not obvious over Giuliano in view of Lee, Sudbo, and Pohl.

Accordingly, claims 1-3 and 5 are patentable over the references of record.

Respectfully submitted,

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